

10/11/2001

Dear Laboratory Director or Supervisor:

Recent events provoked intense interest in testing for *Bacillus anthracis* among the general public and among care providers. As a result you may be receiving clinical samples for culture of this organism. Any laboratory holding a current New York State laboratory permit in the category of General Bacteriology should accept and process samples. We offer the following guidance, published in part on our website (<http://www.wadsworth.org/divisions/infdis/bacti/educational.htm>) with respect to testing for *Bacillus anthracis* in the clinical microbiology laboratory.

**Summary:**

*B. anthracis* should be ruled out 1) in specimens submitted specifically to "rule out anthrax" (e.g., nasal swabs submitted from patients thought to be exposed to aerosols or powders containing *B. anthracis* spores; non-formalinized biopsies of eschar tissue) 2) when *Bacillus* is isolated from a normally sterile body site; 3) when *Bacillus* is the predominant organism in other clinical isolates.

Clinical specimens submitted for *B. anthracis* testing should be processed in a biosafety cabinet at Biosafety Level 2 in clinical laboratories.

Environmental specimens thought likely to contain *B. anthracis* should be processed at Biosafety Level 3 in an experienced Biosafety Level 3 laboratory such as ours after consultation with public health and law enforcement authorities.

**Existing capacity and need:**

As an addendum to the January 25, 2000 Bacteriology - General Proficiency Test, we included the question: "When your laboratory identifies an isolate as *Bacillus* species, do you also rule out *B. anthracis*?" The results of this survey of participating laboratories are listed below:

Rule out *B. anthracis* – 72 (29%)

ID *Bacillus*, but do not rule out *anthracis* – 109 (44%)

Do not ID *Bacillus* – 66 (27%)

The results of this survey on *Bacillus* identifications create the opportunity for laboratories to rethink their current methodology. Human anthrax is endemic in parts of the U.S., Russia, and South Africa. This disease usually occurs from exposure to infected animals or their carcasses or from working industrially with the processing of wool, hair, bones, or other animal products. It is a possibility that an infected patient could be encountered anywhere in the U.S. due to the extent of travel that occurs in this country and internationally. It is also widely believed by many that it is inevitable that a major bioterrorist event may occur in the next decade. The government has acknowledged this possibility by proposing to spend a budget of \$230 million in 2000 on bioterrorism preparedness<sup>1</sup>. In the event that a bioterrorist attack does occur, one of the agents that may be utilized is *Bacillus anthracis*. It is important to note that it is the medical and public health communities that will represent our "first response" in such an incident. The former U.S. Secretary of Health and Human Services, Donna E. Shalala, recently challenged that if we as a country want to be truly prepared for a bioterrorist incident, the medical and public health communities need to take the lead in our fight against bioterrorism.<sup>1</sup> As leaders of the public health community in New York State we encourage laboratories to begin this fight by ruling out anthrax as part of their general laboratory practices.

**Description**

*Bacillus* species are frequently encountered in clinical laboratories and often little effort is made to identify them because they are usually accepted as contaminants with no clinical significance. It is our goal to describe *Bacillus anthracis* and provide uncomplicated means by which laboratories can rule out this

organism. *Bacillus anthracis* are facultative or aerobic, spore-forming bacteria that are part of the *Bacillus* morphological group 1 described by Parry et al.<sup>2</sup> The defining characteristics of this group include sporangia (bacterial cells) that are not swollen or only very slightly swollen by the spores. The spores of this organism are ellipsoidal and are located central to subterminal in the sporangia. *Bacillus anthracis* is Gram-positive although occasionally Gram variability is reported. Additionally, *B. anthracis* are nonmotile, non-hemolytic and make lecithinase, which is visualized as a white halo surrounding colonies on egg-yolk agar. Unlike many *Bacillus* species, wild-type *B. anthracis* are susceptible to penicillin; the possibility exists that strains resistant to penicillin and other antibiotics have been genetically engineered.

The following is specific information on how to best rule out *Bacillus anthracis*. This information was summarized from the ASM Manual of Clinical Microbiology<sup>3</sup> and APHL's Laboratory Protocols for Bioterrorism Response for the Identification of *Bacillus anthracis*.<sup>4</sup>

### **Recommended Precautions:**

Clinical specimens: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures of *B. anthracis*.<sup>5</sup> If *B. anthracis* is suspected a Biosafety Level 2 hood should be utilized.

Suspected bioweapons and other environmental samples: Biosafety Level 3 practices, containment equipment, and facilities are recommended for work involving production quantities or concentrations of cultures. "Production quantities" refers to large volumes or concentrations of infectious agents considerably in excess of those typically used for identification and typing activities.<sup>5</sup> This would include materials such as a powder or aerosol thought possibly to contain large quantities of anthrax spores; these materials should be routed to the Wadsworth Center or another appropriate referral laboratory after consultation with law enforcement and public health authorities.

### **Initial processing:**

Swabs should be plated directly on sheep's blood agar and may also be cultured in an enriched nutrient broth such as trypticase soy or brain heart infusion; the latter can be plated onto sheep's blood agar if cloudy at the end of one or two days' incubation .

**Gram-stain morphology:** Large gram-positive rods (1-1.5 x 3-5 microns) which form oval, central to subterminal spores (1 x 1.5 microns) that do not cause swelling of the cell.

*Spores are not present in clinical samples unless exposed to atmospheric levels of CO<sub>2</sub>. Vegetative cells seen on Gram-stain of blood and impression smears are in short chains of 2-4 cells which are encapsulated.*

**Colonial Characteristics:**After incubation on 5% sheep blood agar plates for 15-24 hours at 35-37°C, well isolated colonies of *B. anthracis* are 2-5 mm in diameter. The colonies are generally flat or slightly convex with an irregularly round shape. The edges are slightly undulate and have a ground glass appearance. There are often comma-shaped projections from the colony edge producing the "Medusa head" colony.

Colonies on sheep blood agar usually have a tenacious consistency. When teased with a loop, the growth will stand up like beaten egg white. ***B. anthracis* are not b-hemolytic** in contrast to *B. cereus* and *B. thuringiensis* colonies. It is possible that some weak hemolysis may be observed under areas of confluent growth in aging cultures.

**Motility Test:** It is important to determine if a *Bacillus* species is motile or nonmotile because ***B. anthracis* is a nonmotile species** whereas most other *Bacillus* species are motile. This test is very useful in the preliminary identification of *B. anthracis* isolates. Either a wet mount motility test or a tubed motility test can be utilized.

**Penicillin susceptibility test: Most *B. anthracis* isolates are susceptible to penicillin.** Penicillin susceptibility testing should be used for organism identification purposes only. The appropriate reagents include 10 unit penicillin susceptibility discs [Remel (1-800-255-6730) or Becton Dickinson/BBL (1-800-675-0908)], nonselective medium (such as 5% sheep blood agar, Mueller-Hinton agar, or trypticase soy agar), and control strains: *Bacillus cereus* ATCC 1586 (Penicillin-resistant) and *B. anthracis* Sterne strain (penicillin susceptible). Sterne strain (avirulent *B. anthracis* vaccine strain): available from Colorado Serum Company, Denver, CO (1-800-525-2065, catalog # 19-102/10 dose or # 19-104/50 dose).

This test should be performed with fresh cultures of the control strains using the same methods as with the unknowns. The control strains should be assayed on each day of testing. If the controls appear to be giving results that are out of range repeat the test with a new lot of penicillin discs and/or verify the purity and identity of the control strains.

**Procedure:**

- Deliver 5 drops (approximately 250 µl) of sterile distilled water into a sterile glass tube.
- Using an inoculating loop, sample a suspicious colony from a 18-24 hour culture and suspend the growth in water.
- Immerse the inoculating swab into the suspension and drain off the excess inoculum by carefully twisting the swab against the inside wall of the tube. Work gently to minimize the production of aerosols.
- Using the inoculating swab, spread the suspension over approximately one-half of the culture plate. Cover the plate and allow it to sit on the bench top for a few minutes to dry.
- Using a pair of forceps, place the penicillin disc in the middle of the inoculated area.
- Incubate the plate aerobically at 35-37° C for 18-24 hours. Examine for the presence or absence of a zone of inhibition. A zone of inhibition may be evident after 4 to 6 hours of incubation.

**Interpretation of Results:**

- Any inhibitory zone is indicative of penicillin susceptibility; however, most *B. anthracis* isolates will exhibit a zone of >20 mm in diameter. Most *B. cereus* isolates will show no zone of inhibition.
- This test should not be used alone in the identification of *B. anthracis* isolates. *Bacillus* isolates of other species may be susceptible to penicillin and penicillin resistance may occur naturally in *B. anthracis* or by genetic manipulation.

**Egg yolk reaction:** If egg yolk agar is available, this test can additionally be utilized. *B. anthracis* synthesizes lecithinases (lecithinase positive egg yolk reaction), forming opaque zones of precipitation around colonies on egg yolk agar.

**Presumptive Identification Key for *B. anthracis*:**

Gram-positive, broad rod, spore-positive: *Bacillus* species

Spores are nonswelling and oval shaped: *Bacillus* morphology group 1 (includes *B. anthracis*, *B. cereus*, *B. thuringiensis*, and *B. cereus var mycoides*) has ground glass appearance of colonies

Nonmotile: *B. anthracis* and *B. cereus var mycoides*

Nonhemolytic, penicillin-sensitive, forms capsule and are lecithinase positive for egg yolk reaction:  
Presumptive *B. anthracis*

*Specimens which are non-motile, non-hemolytic and either have a positive lecithinase reaction or have not been tested for lecithinase should be referred to Wadsworth Center for confirmatory testing. Please call the Bacteriology Laboratory at 518-474-4177 and advise us prior to sending a specimen for confirmation.*

**Specimens:** Nasal swabs are appropriate for screening individuals when a recent exposure to aerosolized *B. anthracis* spores is suspected. We recommend that *B. anthracis* also should be ruled out when *Bacillus* is found as a predominant organism in other clinical specimens received by the bacteriology laboratory. Environmental specimens should be referred to Wadsworth Center after consultation with law enforcement and public health authorities as noted above.

**\*\*** Preserve original specimens pursuant to a potential criminal investigation and forward presumptive isolates to NYSDOH Bacteriology Laboratory immediately. Consultation with NYSDOH Bacteriology Laboratory is strongly encouraged as soon as *B. anthracis* is suspected.

**Contact numbers:**

The Wadsworth Center Bacteriology Laboratory can be reached at 518 474-4177 during normal business hours and through the Department's duty officer at 518 465-9720 during non-business hours.

1 Shalala, D.E. 1999. Bioterrorism: How Prepared Are We? *Emerg. Infect. Dis.* **5(4)**: 492-493.

2 Parry, J.M., P.C.B. Turnbull, and J.R. Gibson. 1988. *A Colour Atlas of Bacillus Species*. Wolfe Medical Publications, London, United Kingdom.

3 Weyant, R.S., Ezzell, J.W., and T. Popovic. 1999. Laboratory Protocols for Bioterrorism Response Laboratories for the Identification of *Bacillus anthracis*. APHLnet.org.

4 Logan, N.A. and P.C.B. Turnbull. 1999. *Bacillus and Recently Derived Genera*, p. 357-369. In Murray P.R., Baron E.J., Pfaller M.A., Tenover F.C., and R.H. Tenover (ed.) *Manual of Clinical Microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.

5 BMBL Section VII Agent Summary Statements Section VII-A: Bacterial Agents. (1999) Biosafety in Microbiological and Biomedical Laboratories (BMBL) - 4th edition. Retrieved March 28, 2000 from the World Wide Web: .